



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

#10

Application of: Cartens, Carsten-Peter

Serial No.: 09/492,590

Examiner: G. Leffers Jr.

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Group: 1636

Entitled: METHODS AND COMPOSITIONS FOR
HIGH LEVEL EXPRESSION OF A
HETEROLOGOUS PROTEIN WITH POOR
CODON USAGE

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Assistant Commissioner for Patents
Washington, D.C. 20231

RULE 132 DECLARATION OF MARY BUCHANAN

I, Mary Buchanan, hereby declare that:

1. I am employed by Stratagene, the assignee of record of the above-referenced patent application. I hold the position of Director of Product Management at Stratagene.

2. Stratagene's CodonPlusTM products contain tRNA genes corresponding to rarely used codons. Stratagene presently sells six different BL21-CodonPlusTM bacterial strains for use in enhancing the expression of protein encoded by genes containing rarely used codons. Bacterial strains BL21-CodonPlusTM RIL (Catalog # 230240), BL21-CodonPlusTM (DE3)-RIL (Catalog # 230245), and BL21-CodonPlus (DE3)-RIL-X (Catalog # 230265), are embodiments of the invention claimed in claims 1-12 and 37. Each of the above competent cell products contains three tRNA genes corresponding to rarely used codons consisting of argU, ileY, and leuW. Bacterial strains BL21-CodonPlusTM RP (Catalog # 230250), BL21-CodonPlusTM (DE3)-RP (Catalog # 230255), and BL21-CodonPlus(DE3)-RP-X (Catalog # 230275), are embodiments of

the invention claimed in claims 39-41 and 44. Each of the above competent cell products contains two tRNA genes corresponding to rarely used codons consisting of argU and proL.

CodonPlusTM host cell products were the first commercially available compositions to successfully allow high level expression of genes encoding rarely used codons. Sales figures provided below indicate the commercial success of these embodiments of the claimed invention (Table I). The products became available in May 1999 in the United States and continue to sell today.

Table I

<u>Year</u>	<u>Catalog #</u>	<u>Product</u>	<u>Number of tRNA genes</u>	<u>Date Introduced</u>	<u>Total Sales</u>
1999	230240	BL21-CodonPlus TM -RIL Competent Cells	3	5/24/99	\$ 103,482
1999	230245	BL21-CodonPlus TM (DE3)-RIL-Competent Cells	3	5/24/99	325,143
1999	230250	BL21-CodonPlus TM -RP Competent Cells	2	8/27/99	18,764
1999	230255	BL21-CodonPlus TM (DE3)-RP Competent Cells	2	8/27/99	46,052
1999 Total					493,441
2000	230240	BL21-CodonPlus TM -RIL Competent Cells	3	5/24/99	59,265
2000	230245	BL21-CodonPlus TM (DE3)-RIL Competent Cells	3	5/24/99	226,181
2000	230250	BL21-CodonPlus TM -RP Competent Cells	2	8/27/99	51,210
2000	230255	BL21-CodonPlus TM (DE3)-RP Competent Cells	2	8/27/99	144,118
2000	230265	BL21-CodonPlus TM (DE3)-RIL-X Competent Cells	3	1/14/00	5,317
2000	230275	BL21-CodonPlus TM (DE3)-RP-X Competent Cells	2	1/14/00	3,429
2000 Total					489,520
2001	230240	BL21-CodonPlus TM -RIL Competent Cells	3	5/24/99	5,174
2001	230245	BL21-CodonPlus TM (DE3)-RIL Competent Cells	3	5/24/99	17,230
2001	230250	BL21-CodonPlus TM -RP Competent Cells	2	8/27/99	3,377
2001	230255	BL21-CodonPlus TM (DE3)-RP Competent Cells	2	8/27/99	15,610
2001	230265	BL21-CodonPlus TM (DE3)-RIL-X Competent Cells	3	1/14/00	162
2001	230275	BL21-CodonPlus TM (DE3)-RP-X Competent Cells	2	1/14/00	162
2001 Total					41,715
TOTAL:					\$ 1,024,676

The primary reason one would use the CodonPlusTM series host cells is to increase the expression of genes containing low frequency codons. The CodonPlusTM series host cells are sold at \$195 per ml, which is 44.4% higher than the same competent cells without the array of tRNA genes (BL21, Catalog # 200133 and BL21 (DE3), Catalog # 200131). Essentially all of the customers who purchased the CodonPlusTM cells used them for the purpose of increasing the expression of heterologous genes encoding rarely used codons because there is no reason to use the more expensive CodonPlus host strains for routine expression of genes without low

frequency codon problems. Therefore, the commercial success of the CodonPlus™ series host cells is due to the presence of an array of two or three tRNA genes encoding rarely used codons.

3. The market for bacterial host cells is shared by a number of major companies, including Invitrogen, Promega, Novagen, and Life Technologies. The consumer is thus free to choose a product on the basis of objective principles. The commercial success Stratagene enjoyed in selling CodonPlus™ host cells is not the result of heavy promotion or advertising, nor the result of consumption by purchasers normally tied to Stratagene. Stratagene spent no more on promotion and advertising of the CodonPlus™ host cells than it did on any of the other new competent cell products it sells. For example, XL10-Gold® Ultracompetent cells (Catalog Nos. 200314, 200315 and 200317) were released in May 1997. Sales in 1997 were \$188,474 worldwide, \$592,193 in 1998. Launch costs were similar for both products.

4. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

February 8, 2001

Date


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Director of Product Management

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